Advancing 3D Cell Culture: Automated positive displacement dispensing of MCF7 and HepG2 cells, mixed with PeptiMatrix™ Hydrogel

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Introduction

***In vitro* cell cultures are essential for modelling disease, but traditional 2D cell culture systems fall short in replicating the complex microenvironments of native tissues. This limitation hinders the translation of *in vitro* findings to *in vivo* contexts and contributes to the high attrition rates seen in drug discovery pipelines.**

To address this, 3D cell culture platforms have been developed to more accurately mimic native tissues and provide more biologically relevant data for downstream studies. However, these methods still face challenges in scaling up for high-throughput drug discovery, primarily due to the complex viscosities of 3D matrices and the need for reproducible, low-volume dispensing in high-density plate formats.

SPT Labtech's dragonfly® discovery is uniquely suited to overcoming these challenges, offering precise, accurate, and liquid-class-agnostic positive-displacement dispensing. In this study, we demonstrate the use of the dragonfly discovery to accurately and evenly dispense MCF7 breast cancer and HepG2 hepatocarcinoma cells, mixed with PeptiMatrix™ hydrogel. PeptiMatrix™ is a synthetic, animal-free peptide hydrogel that can be customized for varying stiffness and composition to more closely model a wide range of tissue types.

Materials and Methods

Cell encapsulation

For each preparation, 1.5 mL of PeptiMatrix™ Core hydrogel (PeptiMatrix, PMCORE100) was placed into a 15 mL centrifuge tube and heated at 80°C for 20 minutes to ensure homogeneity. The hydrogel was then transferred to a 37°C water bath whilst the cell suspension was being prepared.

Cells were harvested, counted, and resuspended in cell culture medium [Dulbecco's Modified Eagle Medium (Gibco) + 10% FBS (BioSera) + 1% L-glutamine (Gibco)] at a concentration of 5 x 10^5 cells/mL. To achieve a final volume of 1.875 mL, 375 µL of the cell suspension was added to 1.5 mL of hydrogel. The suspension was then thoroughly mixed by a combined action of reverse pipetting and gentle stirring.

Dispensing

After cell encapsulation, the cell-gel mixture was transferred to a low dead volume reservoir and dispensed at 100 µL per well into a 96-well plate using the dragonfly discovery. A separate cell-gel mixture was also similarly prepared as above and plated manually at 100 µL per well in a 96-well plate. The gels were incubated at 37 °C for 10 minutes before being overlaid with cell culture medium and incubated at 37°C and 5% CO_2 in a humidified atmosphere. Media changes were performed the following day and then every other day during the culture period.

Live cell imaging and detection

For a qualitative assessment of cell viability, gels were washed with Dulbecco's Phosphate Buffered Saline (DPBS; Gibco) and then incubated for 15 min at room temperature in a solution of 40 µM Ethidium homodimer and 20 µM calcein AM (Thermo Fisher Scientific) in DPBS. Images were then obtained using the EVOS Cell Imaging System (Thermo Fisher Scientific).

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Results and Discussion

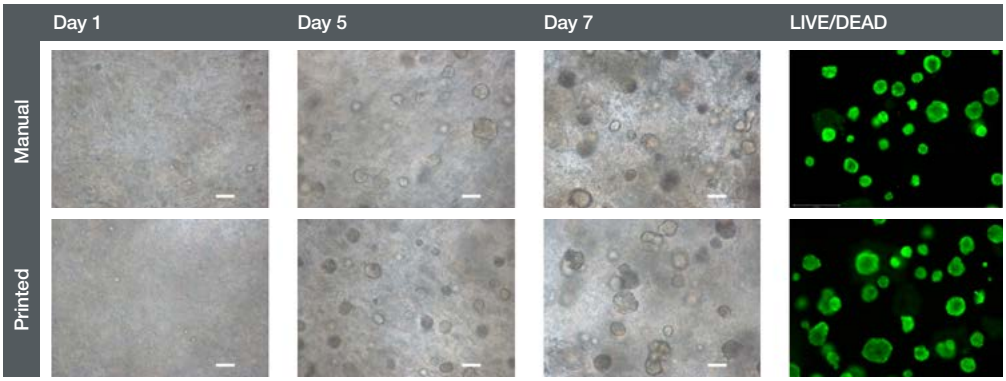

Figure 1. MCF7 breast cancer cells encapsulated in PeptiMatrix™ Core hydrogels and then plated are either by hand pipetting (Manual) or using SPT Labtech's dragonfly discovery (Printed). Shown also is a LIVE/DEAD assay performed on cells on day 7. Scale = 100 µm.

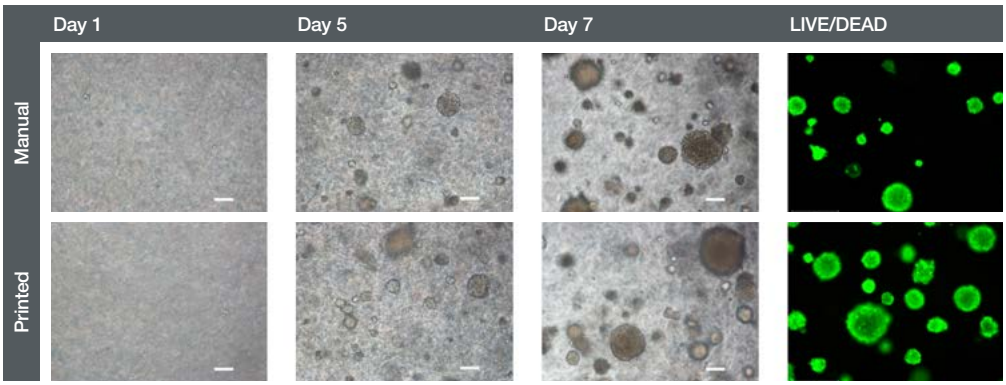

Figure 1. HepG2 hepatocarcinoma cells encapsulated in PeptiMatrix™ Core hydrogels and then plated are either by hand pipetting (Manual) or using SPT Labtech's dragonfly discovery (Printed). Shown also is a LIVE/DEAD assay performed on cells on day 7. Scale = 100 µm.

To successfully automate 3D cell culture assays, liquid handlers must not only be capable of dispensing hydrogels accurately but also consistently. Additionally, it is essential to maintain cell viability during dispensing, ensuring that the resulting spheroids or organoids retain their functional integrity for reliable downstream assays. Consistency in both dispensing and cell behavior is key to achieving reproducible and meaningful results.

Based on results from three independent repeats, Figures 1 and 2 show consistent evidence that both MCF7 and HepG2 cells maintain their characteristic spheroid-forming behavior following dispensing by both hand pipetting and dragonfly discovery:

- Cells dispensed by both methods showed similar viability and continued to form spheroids, with comparable growth rates and spheroid sizes.
- No significant difference was observed in their ability to grow, self-organize, or maintain characteristic spheroid formation.
- Transitioning to an automated dispensing method does not impact cell functionality, while offering benefits in terms of reproducibility and dispensing speed.

Conclusions

Automating the handling of spheroids and organoids is crucial for realizing the full potential of 3D cell culture methods in *in vitro* modelling, tissue engineering, and regenerative medicine.

Advantages of the Integrated Approach

- SPT Labtech's dragonfly discovery, a non-contact positive displacement dispenser, is designed to accurately and precisely dispense low volumes of fluids with varying viscosities.
- PeptiMatrix™ is a highly customizable hydrogel that can be tailored to a wide range of stiffnesses and viscosities, mimicking different tissue types, and can be further modified with specific matrix components to replicate particular tissues.
- The integration of these technologies has the potential to enhance both the throughput and relevance of future 3D cell culture models, driving advances in drug discovery and investigative research.

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